DYNAMIC MULTIPLEXED ANALYSIS METHOD USING ION MOBILITY SPECTROMETER

GOVERNMENT RIGHTS

[0001] The invention was made with Government support under Contract DE-AC0676RLO 1830, awarded by the U.S. Department of Energy. The Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0002] The enormous complexity of biological samples (e.g., from proteomics) and the need for both biological and technical analysis replicates impose major challenges for multidimensional separation platforms in regard to both sensitivity and sample throughput. A major potential attraction of the Ion Mobility Spectrometry-Time-Of-Flight-Mass Spectrometry (IMS-TOF MS) platform is the ability to provide separation speeds exceeding that of conventional condense phase separations by orders of magnitude. Known limitations of most typical IMS-TOF MS platforms that impede this attraction include the need for extensive signal averaging due to factors that include significant ion losses in the IMS-TOF interface and an ion utilization efficiency of less than ~1% with continuous ion sources (e.g., ESI).

[0003] A multiplexed IMS-TOF approach has been shown to provide up to 10-fold increase in sensitivity as compared to the conventional signal averaging approach in regard to analysis of peptide mixtures. This sensitivity improvement is based on introduction of multiple ion packets into an IMS drift tube on the time scale of a single measurement in the signal averaging experiment. Each ion packet injection occurs during a constant IMS gate open event. Ion injection process is governed by an extended pseudo-random sequence that mitigates diffusion-driven ion cloud expansion and enables efficient ion accumulation prior to each gate open event. Short (~100 us) IMS gate open events minimize contribution of the ion injection term on IMS resolving power. A complete description of this invention is found in pending U.S. patent application Ser. No. 11/701,752, entitled "Method of Multiplexed Analysis Using Ion Mobility Spectrometer" the contents of which are hereby incorporated by reference in its entirety.

[0004] The need in multiplexing the IMS-TOF is strongly dictated by the total number of analyte molecules delivered to the ion trap (preceding the IMS drift tube) per unit time and by the charge capacity of that trap. Given lower abundance signals, ion trap may remain under filled with ions in the course of IMS separation, implying no need in multiplexing to attain efficient ion utilization. In this case, ion accumulation over the entire IMS separation would be rather beneficial for achieving high sensitivity. For higher abundance ion signals, the ion trap will be over filled with ions in a fraction of IMS separation timescale, thus requiring the purging the trap multiple times throughout a single IMS separation. Therefore, a combination of approaches is needed to maximize instrument sensitivity in analysis of complex samples with broad dynamic range.

[0005] The present invention describes an approach for increasing the dynamic range of a multidimensional IMS-TOF system in analysis of biological samples. The key feature of this invention is that the multidimensional system automatically adjusts to analyte abundances in the course of experiments, providing an ultra-high sensitivity for a variety of biological samples that significantly vary in complexity and dynamic range.

[0006] Additional advantages and novel features of the present invention will be set forth as follows and will be readily apparent from the descriptions and demonstrations set forth herein. Accordingly, the following descriptions of the present invention should be seen as illustrative of the invention and not as limiting in any way.

SUMMARY OF THE INVENTION

[0007] The present invention is an improvement upon the method for multiplexed analysis using ion mobility spectrometer which has been referenced and incorporated earlier in this application. In the present method, the effectiveness and efficiency of the multiplexed method is optimized by automatically adjusting rates of passage of analyte materials through an IMS drift tube during operation of said system. This automatic adjustment is performed by the IMS instrument itself after determining the appropriate levels of adjustment according to the method of the present invention.

[0008] In one embodiment of the invention, the adjustment of the rates of passage for these materials is determined by quantifying the total number of analyte molecules delivered to the ion trap in a preselected period of time, comparing this number to the charge capacity of the ion trap, selecting a gate opening sequence; and implementing the selected gate opening sequence to obtain a preselected rate of analytes within said IMS drift tube. In some embodiments the quantifying step includes performing an initial short IMS-TOF pre-scan to determine an experimental sequence, said pre-scan conducted in a signal averaging mode using constant short accumulation times (<1 ms), and comprising several IMS-TOF separations. Information obtained from this scan is then utilized to create an IMS-TOF vector from data obtained in this pre-scan. This vector is then folded and summed to obtain the total number of ions accumulated in the ion trap during the pre-scan.

[0009] Each preselected sequence from a set employed in the experiment is characterized by the number of bits, N, with the total number of gate releases per sequence equal to 2^{N-1} and a total sequence length of 2^N-1. To make durations of different bit sequences similar, accumulation times per sequence are proportionally increased with a decrease in the sequence bit number. Let us elucidate this with a simple example. A 6-bit sequence has $2^6-1=63$ modulation bins, each 1 ms long. Accumulation periods throughout the sequence are constant and equal to the shortest interval between two adjacent gate open events. In the case of a 6-bit sequence, each accumulation period equals to 1 ms and the sequence duration is 63 ms. A 5-bit sequence has $2^5-1=31$ modulation bins. To compensate for the 2-fold decrease in the number of modulation bins, the duration of each bin is then increased to 2 ms. Therefore, the duration of a 5-bit sequence is 62 ms, while each accumulation period is 2 ms. Using similar logic, we would find that e.g., a 4-bit sequence is characterized by 4-ms long accumulation periods with the sequence duration of 60 ms.

[0010] Using a calibration function, the total number of ions measured in the pre-scan is converted to the optimum accumulation period of the ion trap. The optimum accumulation period is then compared against the accumulation periods of the preselected pseudo-random sequences and a sequence with the accumulation period closest to the calcu-